



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

FESetup

Citation for published version:

Loeffler, HH, Michel, J & Woods, C 2015, 'FESetup: Automating Setup for Alchemical Free Energy Simulations', *Journal of Chemical Information and Modeling*, vol. 55, no. 12, pp. 2485-90.
<https://doi.org/10.1021/acs.jcim.5b00368>

Digital Object Identifier (DOI):

[10.1021/acs.jcim.5b00368](https://doi.org/10.1021/acs.jcim.5b00368)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Journal of Chemical Information and Modeling

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

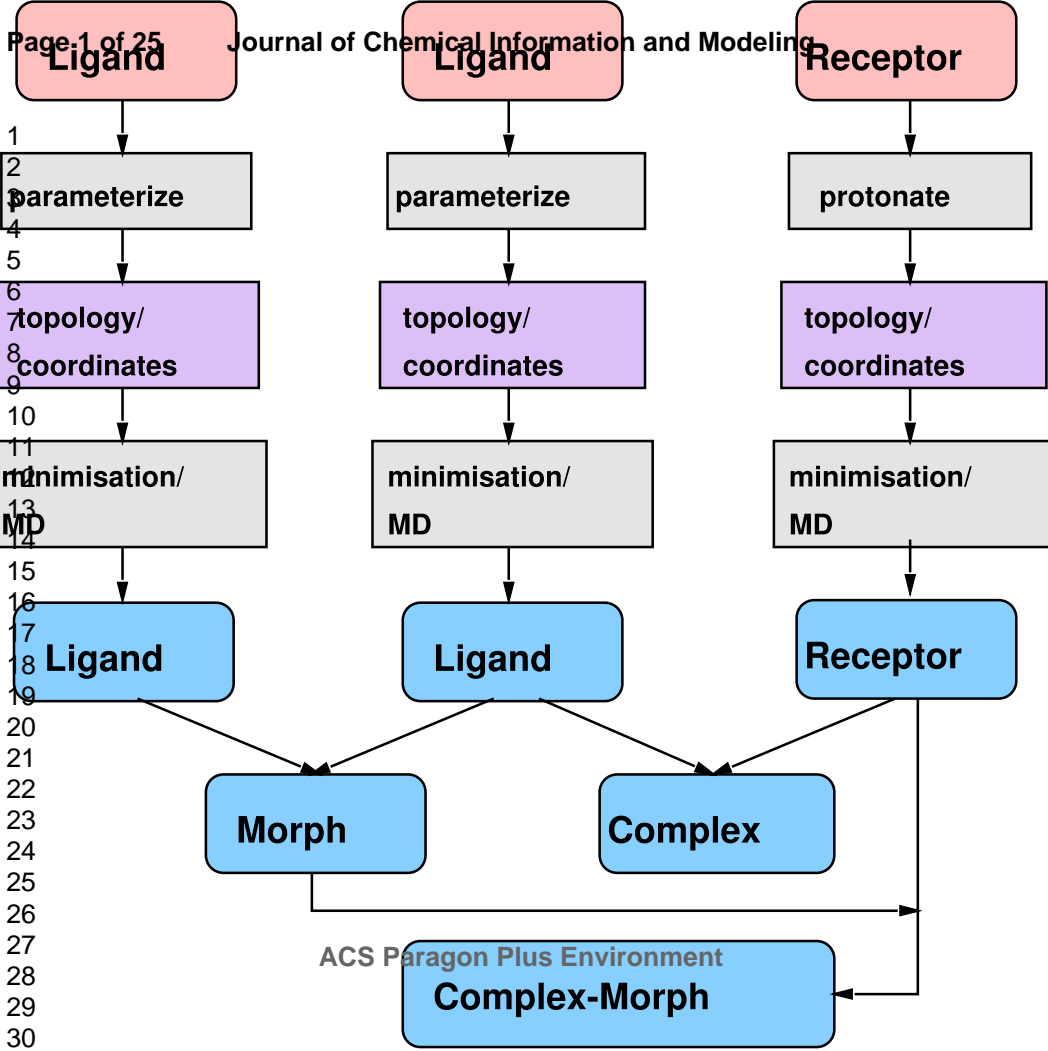


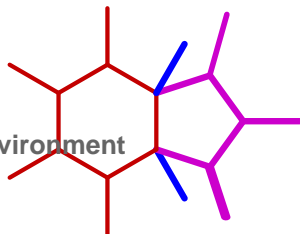
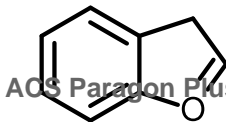
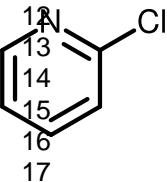
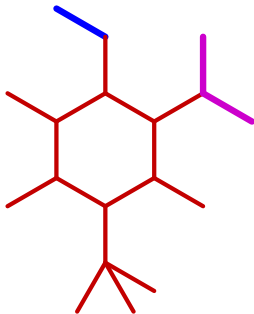
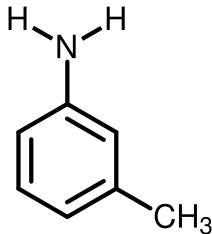
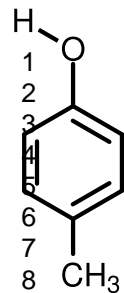
This document is confidential and is proprietary to the American Chemical Society and its authors. Do not copy or disclose without written permission. If you have received this item in error, notify the sender and delete all copies.

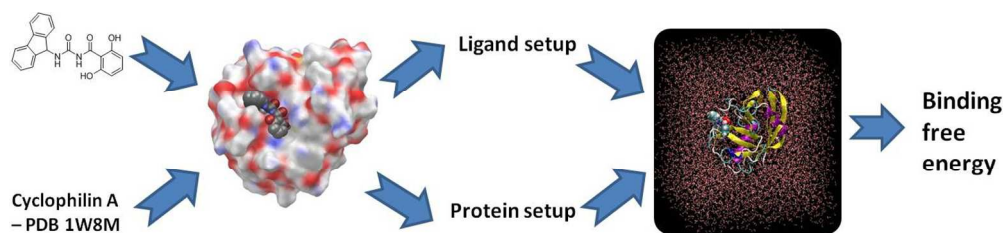
FESetup: Automating Setup for Free Energy Simulations

Journal:	<i>Journal of Chemical Information and Modeling</i>
Manuscript ID	ci-2015-00368y.R1
Manuscript Type:	Application Note
Date Submitted by the Author:	n/a
Complete List of Authors:	Loeffler, Hannes; STFC Daresbury, Scientific Computing Department Michel, Julien; University of Edinburgh, EaStCHEM School of Chemistry Woods, Christopher J.; University of Bristol, BrisSynBio

SCHOLARONE™
Manuscripts







246x58mm (150 x 150 DPI)

FESetup: Automating Setup for Alchemical Free Energy Simulations

Hannes H Loeffler^{1,*}, Julien Michel^{2,‡}, Christopher Woods^{3,‡}

¹Scientific Computing Department, STFC Daresbury, Keckwick Lane, Warrington WA4 4AD,

UK; E-mail: Hannes.Loeffler@stfc.ac.uk

²EaStCHEM School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, UK

³BrisSynBio, University of Bristol, 8–10 Berkeley Square, Bristol BS8 1HH, UK

ABSTRACT: FESetup is a new pipeline tool which can be used flexibly within larger workflows. The tool aims to support fast and easy setup of alchemical free energy simulations for molecular simulation packages such as AMBER, GROMACS, Sire or NAMD. Post-processing methods like MM–PBSA and LIE can be set up as well. Ligands are automatically parametrized with AM1–BCC and atom mappings for a single topology description are computed with a maximum common substructure search (MCSS) algorithm. An abstract

molecular dynamics (MD) engine can be used for equilibration prior to free energy setup or standalone. Currently, all modern AMBER force fields are supported. Ease of use, robustness of the code and automation where it is feasible are the main development goals. The project follows an open development model and we welcome contributions.

Introduction

The setup and input preparation for simulation has become an increasingly demanding task for individual users. While many traditional work-flows are still being managed manually, today's hardware capabilities allow the computation of vast amounts of data. For instance, a system of ca. tens of thousands of atoms can easily be simulated by classical molecular dynamics for at least tens of nanoseconds per day. Such small MD simulations would typically run most efficiently on just a handful of CPU cores while modern hardware may have many thousands of cores available. GPU and other accelerators have increased in popularity in recent times and have tremendously enhanced computing power too especially for small scale installations. Thus, there is a trend for carrying out a multitude of simulations in parallel allowing for large scale comparative studies. It is also important to improve on reproducibility of simulation protocols as manual setups are often poorly documented.

This necessarily means that numerous input files and control data needs to be created to allow the running of large numbers of simulations. However, creating the necessary input files for a simulation can be a laborious and time-consuming process. This was not a problem when simulation run times vastly exceeded manual setup times. However, we are now at a stage where

1
2
3 manual setup is becoming a bottleneck, particularly when running large numbers of simulations.
4
5 It follows there is a growing need for automated setup tools that can minimize user time. The
6
7 key here is to automate work-flows for simulation setup to the maximum extent reasonable. Of
8
9 course, not every step will be easily amenable to automation for various reasons including “hard”
10
11 problems in science such as computing missing structural data from insufficient information and
12
13 limited development of present day algorithms. However, the goal must still be to automate
14
15 what is possible yet at the same time accept that automatic procedures may not always be
16
17 successful. Robust protocols are needed to minimize failure and, importantly, detect and handle
18
19 or report failures.
20
21
22
23

24 Relative alchemical free energy simulation¹ is one example where popular simulation packages
25
26 still offer limited support for setup. This process is very tedious, time-consuming and can easily
27
28 lead to errors as the user may have to edit dozens of files or more and reorder hundreds of lines
29
30 of input by hand. Alchemical free energy simulations certainly require considerably more
31
32 computing resources than e.g. docking methods so this may be one reason for limited uptake
33
34 within the simulation community. However, also the aforementioned obstacles met during setup
35
36 may deter potential users. Therefore, alchemical simulation setup is an interesting target for
37
38 automated simulation setup, especially considering its potential role in drug design and lead
39
40 optimization^{2a}.
41
42
43
44

45 Several attempts at automating the setup of free energy calculations have been reported
46
47 recently. The Free Energy Workflow (FEW) tool² is available for AMBER³ for the setup of
48
49 relative free energy simulations and the end-point methods MM-PB/GBSA⁴ and LIE⁵. Another
50
51 approach which supports AMBER 11 has been reported recently⁶. PMX is a program⁷ which
52
53 automates the setup of relative free energy simulations of side-chain mutations for GROMACS⁸,
54
55
56
57
58
59
60

while StaGE⁹ supports absolute hydration or binding free energy calculations with GROMACS. LOMAP is another software project¹⁰ that does not directly provide simulation input data. LOMAP reduces the graph of all possible ligand pairs (relative free energies) to a minimum set based on a definition of similarity used to weight the graph's edges to solve the shortest path tree problem. The binding affinity calculator¹¹ (BAC) is an automation tool for rapid computation and analysis of ligand–receptor free energies using MM–PB/GBSA.

Here, we will discuss a new tool called FESetup. FESetup's advance over previous tools is that it is designed to support alchemical free energy simulations in a range of different MD and Monte Carlo (MC) packages. FESetup presents an abstraction of the setup process that is independent of a given MD or MC code, and that is flexible enough to work within larger workflows e.g. using docking software to provide receptor–drug structures. FESetup has been built to be open source and flexible, providing a strong foundation to build setup workflows for different free energy methods. Further goals are support for mutation of both non–covalently and covalently bound moieties, maximal automation, robustness, the development of an API and documentation of all outputs in a log file. In this Note, we will summarize current progress and how FESetup can help the user to set up free energy calculations for codes like AMBER, GROMACS, Sire¹² and also NAMD¹³. FESetup is free software (GPL) and is installed locally. The project is developed in an open fashion where interested parties can contribute at all levels including code contributions and interfacing to FESetup.

Methodology

Absolute vs Relative

There are principally two ways to compute alchemical free energies along a coupling parameter λ ¹⁴: the *absolute* and the *relative* approach. *Absolute* free energy changes are obtained

by completely annihilating e.g. a ligand in solution¹⁵ and in the bound state (which yields an absolute binding free energy), or by annihilating a ligand in different solvents (which yields an absolute solvation or transfer free energy). The setup for this type of alchemical calculation in modern packages like AMBER or GROMACS is very easy. Both programs allow the user to tag a set of atoms for decoupling through specific keywords in the input file. Both also allow some flexibility for separating the van-der-Waals free energy path from the electrostatic free energy path. The user is *not* required to modify the topology directly as this is transparently done in-code.

Single vs Dual-Topology

For *relative* free energy simulations there is a choice to be made between the single-topology and the dual-topology approach^{16,17}. Codes like NAMD only allow the latter at the moment and when this approach is applied to non-covalently bound molecules it suffers from the same problem as the absolute approach. The final end states describe a “non-existing” molecule that can freely drift through the receptor and this needs to be compensated for with adequate restraints¹⁸.

Codes like AMBER or GROMACS implement hybrid approaches since they allow the assignment of a single and a dual-topology region at the same time. The single-topology region can serve as a convenient “anchor” to keep the ligands in place as the coordinates are shared and only direct conversion of one atom to another is allowed to occur. The atoms within this region are thus always present.

MCSS mapping

The single-topology approach requires a one-to-one mapping of equivalent atoms. While this is generally a rather simple task for a human operator it requires some care when done

algorithmically. In cases where there is a well-defined anchor region as in protein backbone atoms, the remaining atoms can be mapped through a simple distance criterion⁷. A general solution is needed, however, in the case of arbitrary molecular structures.

Maximum common substructure search (MCSS) algorithms have been used in connection with the definition of a similarity criterion to decide when two atoms or a bond match each other^{6,10}. FESetup uses fmcs¹⁹ which is a connected MCSS algorithm²⁰. Our similarity criterion is that any atom or any bond can match each other but rings must always match rings and rings can't be broken²¹. Hydrogen atoms are explicitly included in the MCS. Our scheme essentially aims for maximum overlap⁷, the idea being that a maximal single-topology description is the most efficient protocol. This also implies that the number of vanishing or appearing atoms ("dummy" atoms) is minimal (see Chart 1). In the definition of ref. 38 this implies an implicit intermediate. The internal representation of molecules is package-independent and thus allows generation of any arbitrary output format.

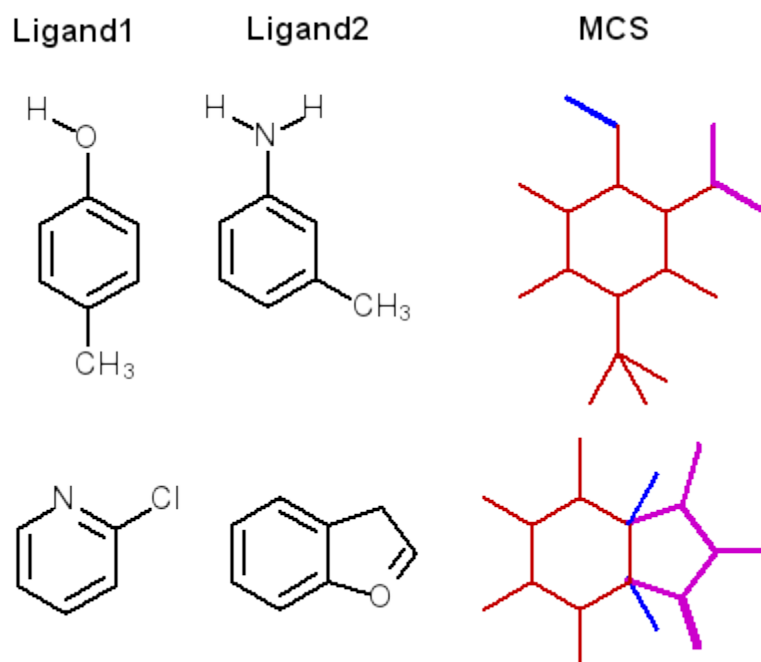


Chart 1. Example for two ligand morph pairs. The MCS (red) is shown with explicit hydrogens on the right together with vanishing (blue) and appearing atoms (magenta).

Two structures can principally have more than one common substructure²⁰. In our scheme, where we ignore atom and bond type identities, even more solutions are possible, e.g. the methanol to ethanol transformation has four unique solutions in terms of our definition of similarity. Different MCSs merely define a different pathway and the free energies obtained from a closed thermodynamic cycle should be independent of the pathway chosen to connect different molecules. However, some pathways may be more computationally efficient than others. This is especially so for ligands binding to a receptor, often involving a high degree of symmetry, where the binding mode may need to be preserved. Although a ligand can, in principal at least, adjust to λ dependent changes, sufficient sampling may be very hard to achieve in practice. The preservation of the binding mode is the responsibility of the user in FESetup.

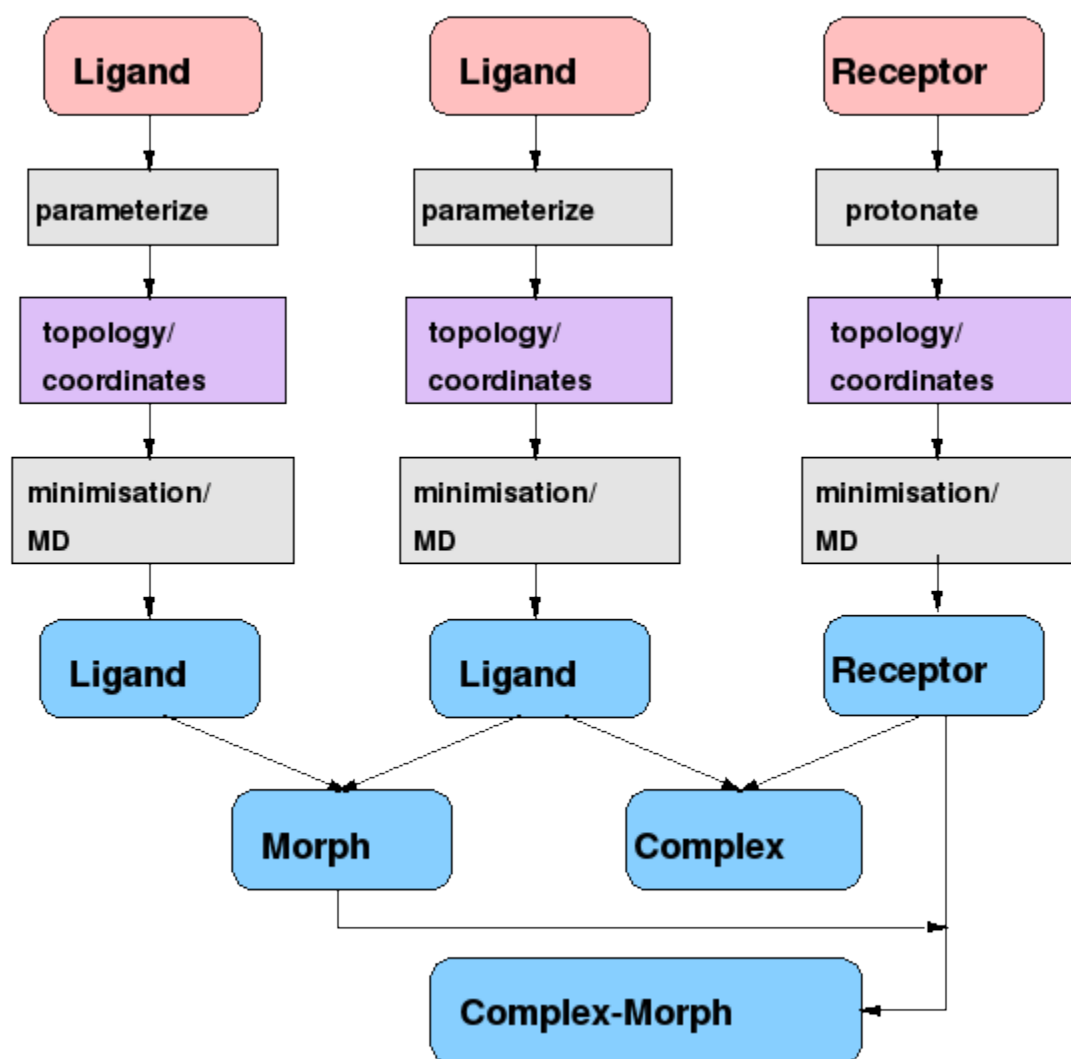
Another issue is chirality because molecular graphs are, by definition, only two-dimensional. This means that chiral centers may be inverted because the MCSS algorithm simply follows the

longest path through the graph. A solution has been proposed earlier⁶ but this removes potential mappings in the R-groups and also the asymmetric carbon.

To handle all these complexities, FESetup allows the user to arbitrarily assign desired mappings through a file mechanism. The user creates a file for each mutation containing pairs of mapping indices for each corresponding atom pair. This is a very general solution that allows overwriting of any choice FESetup would otherwise take.

Workflow

Scheme 1 depicts the principal work flow in FESetup to set up a relative free energy calculation that morphs from ligand 1 to ligand 2 while bound to a receptor. Various third party software and toolkits are recruited for setup. Openbabel²² is used to convert file formats, carry out preliminary minimization and possibly create alternative conformations. The latter can be useful to provide multiple starting structures and could potentially be used to create charges from multiple conformations. RDKit²³ is used to compute the MCS between a ligand pair (a “morph”) with the fmcs algorithm¹⁹. Sire is used to read AMBER topology files and provide data structures for force field parameters and allow for their manipulation. Furthermore, Sire can detect and select internal degrees of freedom for Monte Carlo (MC) simulations. The AmberTools antechamber and leap are used to create AMBER topology files including force field parameterization.



Scheme 1. The principal work flow in FESetup. Optional steps are in gray boxes. The pink boxes signify input structures and blue boxes ready simulation systems.

The work flows for ligand and receptor are independent from each other and arbitrary numbers of either can be run. The ligand is automatically parametrized with antechamber but users can supply parameters of their own. FESetup supports currently all modern AMBER force fields for biomolecules, lipids and carbohydrates. For the ligand the default force field is GAFF²⁴ but this can be overwritten, e.g. for a previous free energy study on carbohydrates²⁵ we have used the GLYCAM force field²⁶. By default atomic partial charges are derived with the AM1–BCC method²⁷.

The semi-empirical AM1-BCC charges are derived with sqm, the semi-empirical quantum mechanics code used to compute the atomic partial charges, which is supplied with AmberTools. The default convergence criteria are very tight, however. FESetup will reduce these criteria when SCF convergence fails. As part of this, the MMFF94 force field may be used to slightly modify the starting coordinates. This has been found to facilitate convergence in sqm in some cases. The final charges are written by antechamber with 4 digit precision only which can lead to rounding errors and a non-integer net charge. The code redistributes this charge to a higher precision evenly among all atoms to match the total molecular charge.

Protonation, and more specifically the tautomeric, state of the ligand is the responsibility of the user. A recent paper has pointed out the inherent problems of assigning these automatically with chemoinformatic tools²⁸. Total charges are communicated through the input structure file. Openbabel will read these from e.g. the PDB and SDF formats but *not* the popular MOL2 format. The latter format is typically used with the antechamber tool chain.

After parametrization, leap is recruited to create topology and coordinate files for either vacuum or a solvated simulation box with counter ions. Preset minimization and MD protocols can be carried out to adjust the density and provide starting velocities via an abstraction interface that supports a number of MD engines: AMBER, GROMACS, NAMD or DL_POLY²⁹. Topologies and coordinates will be transparently converted to GROMACS and DL_POLY formats (but alchemical free energy simulations are not yet supported for the latter). The direct conversion has the advantage that the newest AMBER force fields are always immediately available independent of a native port to a particular MD package.

1
2
3 The receptor can optionally be protonated with PROPKA^{30,31} but otherwise the work flow is
4 the same as for the ligand. The receptor can optionally be aligned along the principal axes. Any
5 biomolecule which is supported by the AMBER force fields and leap may be used.
6
7

8
9
10 Two ligands can be combined into a morph pair. FESetup will determine the maximum
11 common substructure and use this to set up the mapped region with a set of common coordinates.
12 Any atoms not mapped in this way are described as softcore (dummy) atoms. The code will
13 create all necessary topology and coordinate files for the perturbed simulation for the MD
14 packages AMBER, GROMACS and Sire. NAMD is principally supported too because NAMD
15 can read AMBER files but it is dual-topology only (see discussion above).
16
17
18
19
20
21
22
23

24 The receptor can be combined with a complex to form a complex-morph. The solvated box is
25 created from the coordinates of the unperturbed simulation system. The only additional atoms
26 and their coordinates are those for the dummy atoms. These will be computed from the internal
27 coordinates of the other state with the existing atoms. This is not necessary for AMBER³²
28 because AMBER topologies can be created without explicit dummy atoms. The assumption is
29 made that all bonded terms involving softcore atoms will cancel in a thermodynamic cycle and
30 thus need not be explicitly computed. FESetup provides, however, setup with explicit dummy
31 atoms for AMBER too.
32
33
34
35
36
37
38
39
40
41
42

43 The force field parameters for dummy atoms have to be created except for AMBER which
44 handles this entirely internally. For GROMACS and Sire we follow a scheme of copying the
45 bond and angle force field terms from the respective other state i.e. the end state where the atoms
46 do exist. The end state of a bond or angle that involves dummy atoms has thus the same, non-
47 zero parameters as the original atom. This implies that these bonded parameters of softcore
48
49
50
51
52
53
54
55
56
57
58
59
60

atoms will be independent of the coupling parameter λ . Dihedrals or impropers involving dummy atoms can be set to zero.

Input/Output

FESetup has been designed to be easy to use with little input from the user and with sensible defaults but also at the same time to enable the user to overwrite decisions the software may make. Input is handled through a shell script, called FESetup, which sets up the environment and calls dGprep.py which is the actual Python code handling user input. The input file is in an INI style format very close to the configuration file format as typically used on Microsoft operating systems and is thus easy to understand and parse. The input file is divided into 4 sections: [global], [complex], [protein] (historical synonym for receptor) and [ligand] (historical synonym for unparameterized molecule) sections.

FESetup will create all topology and template control files required for simulation. The input files do not, however, prescribe a specific λ schedule. It is not clear *a priori* what λ path would guarantee a smooth gradient (TI) or sufficient energetic overlap (FEP/BAR). This will depend on the nature of the system and is still an open question.

The *Supporting Information* demonstrate typical input examples and also provide the results for the relative hydration free energies of two test system. Further validation is presented through single point energies of the end-states. Fully worked tutorials can be found on our web page (see below).

API

With FESetup we also define an application programming interface (API). In fact, dGprep.py is an elaborate example of how to use the API. See the *Supporting Information* for details.

Online material

FESetup can be downloaded from <http://www.hecbiosim.ac.uk/fesetup>. The source code repository including a Wiki, tutorials, a manual page, a discussion board, bug tracker and feature tracker are hosted through CCP–Forge on <http://ccpforge.cse.rl.ac.uk/gf/project/ccpbiosim/>. FESetup is developed in Python 2.7 and thus principally highly portable. Restrictions may apply to the third party software. FESetup comes as a pre-compiled, self-installing shell script. Binaries are available for Intel (and compatible) Linux 32 and 64 bit. FESetup is licensed as free software under the GPL2 license.

Applications

FESetup is already cited in a number of published studies and is used in several ongoing studies.

In previous work³⁵ FESetup was used to prepare input files for multiple ligands bound to diverse proteins and in solution. Alchemical free energy calculations were not performed, but FESetup was very useful to automate the setup process and to ensure that a consistent setup protocol was applied throughout. The resulting input files were used to produce molecular dynamics simulation trajectories and these were processed by the software nautilus³³⁻³⁴ to compute enthalpies and entropies of water molecules in diverse protein binding sites using the grid cell theory method.

In another paper²⁵ the usefulness of the specialized carbohydrate force field GLYCAM and the general force field GAFF have been investigated in their application to a lectin complexed with various mono-saccharide pyranoses. Alchemical free energy simulations have shown that GLYCAM mostly outperforms GAFF. FESetup has been used to set up all free energy simulations. The mapping feature proved to be very useful to preserve chirality and maximum mapping at the same time.

Conclusions and Outlook

FESetup is a pipeline tool to make the setup of alchemical free energy simulations easier, faster and less error-prone. The tool can flexibly be integrated into larger workflows taking in a wide variety of structures. It creates simulation input for the MD packages AMBER, GROMACS, Sire, and, to some extent, NAMD. A maximum common substructure algorithm is used to automatically determine one-to-one mappings between start and end states. Ligands are automatically and transparently parametrized to provide AM1-BCC charges.

An abstract MD engine can be used to equilibrate (minimize, heat, pressurize, restraint release) the unperturbed simulation systems. These engines are available for AMBER, GROMACS, NAMD and DL_POLY, and can be used independently from the alchemical setup code. In principle, this can be developed into a general MD driver and combined with job submission code to off-load compute-intensive tasks to a remote HPC system.

FESetup is free software released under the GPL2 license. The code is installed locally which means that no confidential data has to be transferred over a network, is always available and the source code can be inspected and modified. The third party software is freely available too. The project follows an open development model accepting contributions of all kinds.

Future development will focus on expanding functionality to mutation of covalently bound moieties like side-chains, and integration of absolute free energy setup. The goal here is to allow arbitrary transformations just as with the current ligand support. Other popular MD packages (support for the PERT module in CHARMM³⁶ is currently developed) and other popular force fields like CHARMM or OPLS will be supported too. Alternative parametrization schemes like RESP³⁷ can be integrated too as the work-flow is mostly automatic.

ASSOCIATED CONTENT

Supporting Information. Detailed input examples, examples of free energy changes computed with different codes using FESetup generated input files. Table of single point energies for several transformations setup for different simulation packages.

AUTHOR INFORMATION

Corresponding Author

*Hannes H Loeffler, *E-mail:* Hannes.Loeffler@stfc.ac.uk

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Funding Sources

HHL is supported through an EPSRC provided SLA, funding the core support of CCPBioSim. CCPBioSim is the Collaborative Computational Project for Biomolecular Simulation funded by

EPSRC grants EP/J010588/1 and EP/M022609/1. JM is supported by a Royal Society University Research Fellowship. CJW is grateful to the BBSRC (BB/K016601/1) and BrisSynBio for funding. BrisSynBio is a BBSRC/EPSRC-funded Synthetic Biology Research Centre (BB/L01386X/1).

Notes

ACKNOWLEDGMENT

We are grateful to the continuing HPC support through the STFC Scientific Computing Department's SCARF cluster and the STFC Hartree Centre. CCP-Forge (EPSRC grant EP/L000342/1) is acknowledged for hosting the source code repository, the wiki and the tutorials. James Gebbie (HECBioSim) is acknowledged for designing and hosting the front-end page for FESetup and contributions towards the installer. Chin Yong and Ilian Todorov are acknowledged for helping with DL_FIELD and DL_POLY support. Jason Swails is acknowledged for helping with ParmEd.

ABBREVIATIONS

MD, Molecular Dynamics; MCSS, maximum common substructure search; MCS maximum common substructure; AM1, Austin Model 1 Hamiltonian; BCC, bond charge correction; SCF, self-consistent field; API, application programming interface; MC, Monte Carlo;

References

- (1) Michel, J. Current and emerging opportunities for molecular simulations in structure-based drug design. *Phys. Chem. Chem. Phys.* **2014**, *16*, 4465–4477.
- (2) Homeyer, N., Gohlke, H. FEW: A workflow tool for free energy calculations of ligand binding. *J. Comput. Chem.* **2013**, *34*, 965–973.

(2a) Jorgensen, W. L. Efficient Drug Lead Discovery and Optimization. *Acc. Chem. Res.* **2009**, *42*, 724–733.

(3) Case, D. A.; Thomas E., III, C.; Darden, T.; Gohlke, H.; Luo, R.; Merz, Jr., K. M.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R. J. The Amber biomolecular simulation programs. *J. Comput. Chem.* **2005**, *26*, 1668–1688.

(4) Srinivasan, J.; Cheatham, T. E.; Cieplak, P.; Kollman, P. A.; Case, D. A. Continuum Solvent Studies of the Stability of DNA, RNA, and Phosphoramidate–DNA Helices. *J. Am. Chem. Soc.* **1998**, *120*, 9401–9409.

(5) Gutiérrez-de-Terán, H.; Åqvist, J. Linear Interaction Energy: Method and Applications in Drug Design. In *Computational Drug Discovery and Design*; Baron, R., Ed.; Springer New York, 2012; pp 305–323.

(6) Christ, C. D.; Fox, T. Accuracy Assessment and Automation of Free Energy Calculations for Drug Design. *J. Chem. Inf. Model.* **2014**, *54*, 108–120.

(7) Gapsys, V.; Michielssens, S.; Seeliger, D.; de Groot, B. L. pmx: Automated protein structure and topology generation for alchemical perturbations. *J. Comput. Chem.* **2015**, *36*, 348–354.

(8) Van Der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. GROMACS: Fast, flexible, and free. *J. Comput. Chem.* **2005**, *26*, 1701–1718.

(9) Lundborg, M.; Lindahl, E. Automatic GROMACS Topology Generation and Comparisons of Force Fields for Solvation Free Energy Calculations. *J. Phys. Chem. B* **2015**, *119*, 810–823.

(10) Liu, S.; Wu, Y.; Lin, T.; Abel, R.; Redmann, J. P.; Summa, C. M.; Jaber, V. R.; Lim, N. M., Mobley, D. L. Lead optimization mapper: automating free energy calculations for lead optimization. *J. Comp.-Aided Mol. Des.* **2013**, *27*, 755–770.

- (11) Sadiq, S. K.; Wright, D.; Watson, S. J.; Zasada, S. J.; Stoica, I.; Coveney, P. V. Automated Molecular Simulation Based Binding Affinity Calculator for Ligand-Bound HIV-1 Proteases. *J. Chem. Theory Comput.* **2008**, *48*, 1909–1919.
- (12) Woods, C. J.; Michel, J. M. *Sire: An advanced, multiscale, molecular simulation framework*, <http://siremol.org/> (accessed Sep 4, 2015).
- (13) Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kale, L.; Schulten, K. Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **2005**, *26*, 1781–1802.
- (14) Hansen, N., van Gunsteren, W. F. Practical Aspects of Free-Energy Calculations: A Review. *J. Chem. Theory Comput.* **2014**, *10*, 2632–2647.
- (15) Mobley, D., Guthrie, J. FreeSolv: a database of experimental and calculated hydration free energies, with input files. *J. Comp.-Aided Mol. Des.* **2014**, *28*, 711–720.
- (16) Boresch, S., Karplus, M. The Role of Bonded Terms in Free Energy Simulations: 1. Theoretical Analysis. *J. Phys. Chem. A* **1999**, *103*, 103–118.
- (17) Michel, J., Essex, J. Prediction of protein--ligand binding affinity by free energy simulations: assumptions, pitfalls and expectations. *J. Comp.-Aided Mol. Des.* **2010**, *24*, 639–658.
- (18) Gumbart, J. C.; Roux, B., Chipot, C. Standard Binding Free Energies from Computer Simulations: What Is the Best Strategy?. *J. Chem. Theory Comput.* **2013**, *9*, 794–802.
- (19) Dalke, A., Hastings, J. FMCS: a novel algorithm for the multiple MCS problem. *J. Cheminform.* **2013**, *5*, O6.
- (20) Raymond, J. W., Willett, P. Maximum common subgraph isomorphism algorithms for the matching of chemical structures. *J. Comp.-Aided Mol. Des.* **2002**, *16*, 521–533.

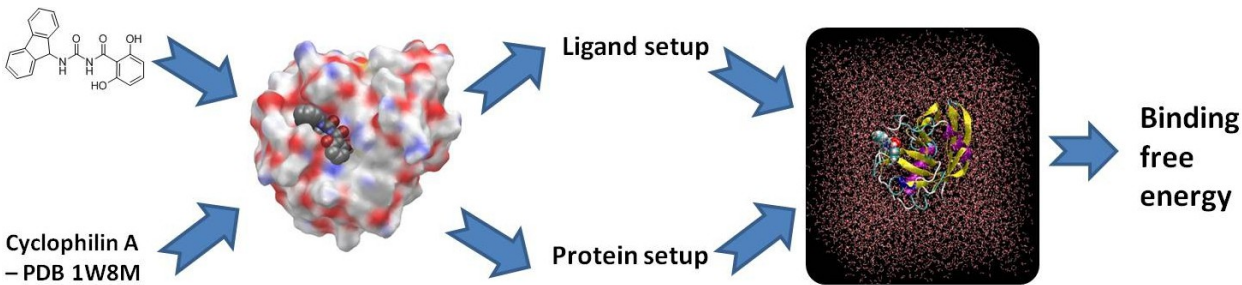
- (21) Liu, S.; Wang, L.; Mobley, D. L. Is Ring Breaking Feasible in Relative Binding Free Energy Calculations?. *J. Chem. Inf. Mod.* **2015**, *55*, 727–735.
- (22) O'Boyle, N.; Banck, M.; James, C.; Morley, C.; Vandermeersch, T.; Hutchison, G. Open Babel: An open chemical toolbox. *J. Cheminform.* **2011**, *3*, 33–46.
- (23) Landrum, G. *RDKit: Open-source cheminformatics*, <http://www.rdkit.org> (accessed Sep 4, 2015).
- (24) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. Development and testing of a general amber force field. *J. Comput. Chem.* **2004**, *25*, 1157–1174.
- (25) Mishra, Sushil K; Calabro, Gaetano; Loeffler, Hannes H.; Michel, J.; Koča, J. Evaluation of selected classical force fields for alchemical binding free energy calculations of protein-carbohydrate complexes. *J. Chem. Theory Comput.* **2015**, *11*, 3333–3345.
- (26) Kirschner, K. N.; Yongye, A. B.; Tschampel, S. M.; González-Outeiriño, J.; Daniels, C. R.; Foley, B. L.; Woods, R. J. GLYCAM06: A generalizable biomolecular force field. Carbohydrates. *J. Comput. Chem.* **2008**, *29*, 622–655.
- (27) Jakalian, A.; Bush, B. L.; Jack, D. B.; Bayly, C. I. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: I. Method. *J. Comput. Chem.* **2000**, *21*, 132–146.
- (28) Sayle, R. So you think you understand tautomerism?. *J. Comp.-Aided Mol. Des.* **2010**, *24*, 485–496.
- (29) Todorov, I. T.; Smith, W.; Trachenko, K.; Dove, M. T. DL_POLY_3: new dimensions in molecular dynamics simulations via massive parallelism. *J. Mater. Chem.* **2006**, *16*, 1911–1918.
- (30) Søndergaard, C. R.; Olsson, M. H. M.; Rostkowski, M.; Jensen, J. H. Improved Treatment of Ligands and Coupling Effects in Empirical Calculation and Rationalization of pKa Values. *J. Chem. Theory Comput.* **2011**, *7*, 2284–2295.

- (31) Olsson, M. H. M.; Søndergaard, C. R.; Rostkowski, M.; Jensen, J. H. PROPKA3: Consistent Treatment of Internal and Surface Residues in Empirical pKa Predictions. *J. Chem. Theory Comput.* **2011**, *7*, 525–537.
- (32) Kaus, J. W.; Pierce, L. T.; Walker, R. C.; McCammon, J. A. Improving the Efficiency of Free Energy Calculations in the Amber Molecular Dynamics Package. *J. Chem. Theory Comput.* **2013**, *9*, 4131–4139.
- (33) Michel, J.; Henchman, R. H.; Gerogiokas, G.; Southey, M. W. Y.; Mazanetz, M. P.; Law, R. J. Evaluation of Host--Guest Binding Thermodynamics of Model Cavities with Grid Cell Theory. *J. Chem. Theory Comput.* **2014**, *10*, 4055–4068.
- (34) Gerogiokas, G.; Calabro, G.; Henchman, R. H.; Southey, M. W. Y.; Law, R. J.; Michel, J. Prediction of Small Molecule Hydration Thermodynamics with Grid Cell Theory. *J. Chem. Theory Comput.* **2014**, *10*, 35–48.
- (35) Gerogiokas, G.; Southey, M. W. Y.; Mazanetz, M. P.; Hefetz, A.; Bodkin, M.; Law, R. J.; Michel, J. Correction: Evaluation of water displacement energetics in protein binding sites with grid cell theory. *Phys. Chem. Chem. Phys.* **2015**, *17*, 16213–16213.
- (36) Brooks, B. R.; Brooks, C. L.; Mackerell, A. D.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S.; Caflisch, A.; Caves, L.; Cui, Q.; Dinner, A. R.; Feig, M.; Fischer, S.; Gao, J.; Hodoscek, M.; Im, W.; Kuczera, K.; Lazaridis, T.; Ma, J.; Ovchinnikov, V.; Paci, E.; Pastor, R. W.; Post, C. B.; Pu, J. Z.; Schaefer, M.; Tidor, B.; Venable, R. M.; Woodcock, H. L.; Wu, X.; Yang, W.; York, D. M.; Karplus, M. CHARMM: The biomolecular simulation program. *J. Comput. Chem.* **2009**, *30*, 1545–1614.

(37) Bayly, C. I.; Cieplak, P.; Cornell, W., Kollman, P. A. A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model. *J. Phys. Chem.* **1993**, *97*, 10269–10280.

(38) Mobley, D. L.; Klimovich, Pavel V. Perspective: Alchemical free energy calculations for drug discovery. *J. Chem Phys.* **2012**, *137*, 230901.

Insert Table of Contents Graphic and Synopsis Here



A graphical workflow of FESetup. A protein from the PDB and a ligand are combined and solvated. Input files are created to carry out binding free energy simulations.